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TITLE: HIGH SPEED SINGLE PARTICLE SIZING BY LIGHT SCATTERING IN A FLOW SYSTEM

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High speed single particle sizing by light scattering in a flow system

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Abstract

We present two approaches to rapid, single particle sizing for particles in the 1 to 20 μm diameter range. One method measures multiangle scattered light over a polar angular range of nearly 360 degrees. A second method is based on the analysis of the pulse shapes from small angle forward scattered light. In both cases the particles in liquid suspension are made to pass one at a time through a focused laser beam for analysis.

Introduction

Light scattering has been used for many years to size distributions of particles in cuvettes. More recently, instruments have been developed in which individual particles in the diameter range from 1 to 20 μm pass in single file through a focused laser beam. The light scattered by a particle is then measured in several ways to determine its size. Some of these tools have evolved from a biomedical research area named Flow Cytometry, which describes measurements on biological cells in flow. Light scattering has played a significant role in the sizing of biological cells.[1]

Here we focus on two techniques for measuring the sizes of individual particles. The first is a flow photometer based on an idea by Gucker and Tuma.[2] The system[3,4] is capable of sampling 60 points in the scattered light pattern of a particle as it passes through a focused laser beam. The second is an electronic pulse shape analysis module for use with any flow photometer having a focused laser beam width comparable to or smaller than the particles of interest.

The 360 degree light scatter flow photometer

Figure 1 shows a schematic drawing of the 360 degree flow photometer. The 488 nm wavelength laser beam with vertical polarization intersects the stream of particles at one focus of an ellipsoidal reflector. Light scattered in the vertical plane over the polar angular range from approximately 4 degrees to 175 degrees and from 185 degrees to 356 degrees is reflected from the ellipsoidal mirror toward a multielement detector array. This 2 cm diameter circular array consists of 60 silicon PIN photodiodes in chip form, mounted on a ceramic substrate. Each element subtends a polar angular range of 2.9 degrees. The signal from each detector is preamplified by a hybrid circuit mounted close to the array. A selected subset of 32 of the signals is logarithmically amplified, digitzed and stored on a computer disk at rates up to 1000 particles/sec.[3,4]

To test the ability of this photometer to discriminate among different size particles, we analyzed a mixture of polystyrene latex microspheres with mean diameters 1.1, 5.0, 10.0, 15.6 and 19.5 μm. The volume coefficients of variation were less than 3%. Figure 2 shows the frequency distributions of the scattered light intensities for the first eight detectors in the lower half plane. The ordinate is the number of particles and the abscissa indicates 3 decades of logarithmic scattered light intensity. Table 1 gives the relationship between detector number and detector angle and also indicates which particle sizes are resolved at each detector. At detectors 1 through 4 only the 1.1 μm and 5.0 μm diameter particles are on scale. At detector 8 the 4 larger sizes are resolved and the 1.1 μm diameter particles are buried in signals from noise and debris.

Particle sizing by pulse shape analysis

The shape of the detected scattered light signal as a particle passes through a focused laser beam can be used to measure the particle dimension parallel to the detection of flow.

Figure 3 shows some of the measurements which can be made on this waveform. We focus attention on only two of these in this report. CFRT(F1-F2) is the constant fraction rise-time of the leading edge of the pulse between fixed fractions F1 and F2 of the pulse height

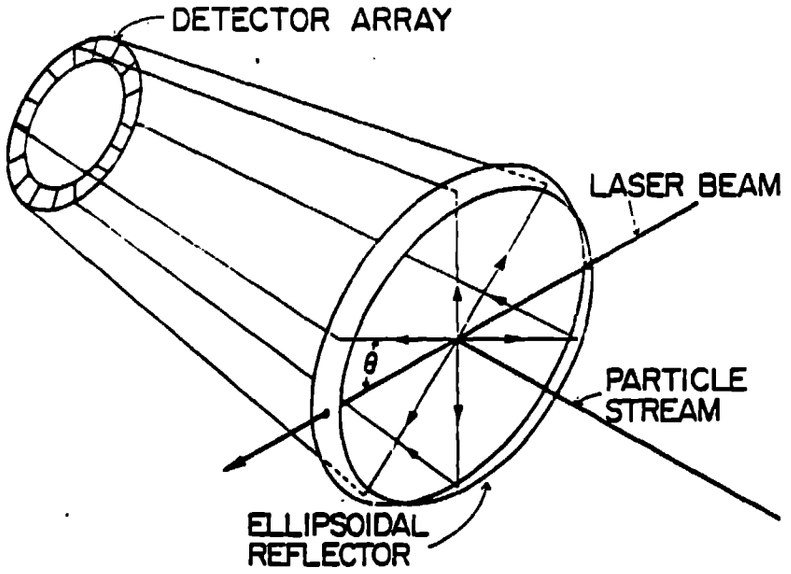

Figure 1. Schematic drawing of the 360 degree flow photometer. The section of ellipsoidal reflector is enclosed in a fluid-filled chamber with a quartz window facing the detector array.

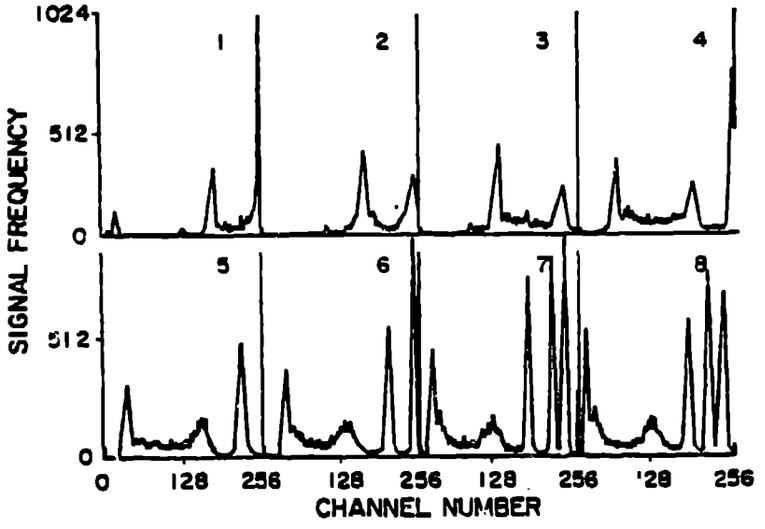

Figure 2. Frequency distributions of the scattered light intensities for latex microspheres for the first 8 detector elements in the forward direction. The ordinate shows number of particles and the abscissa shows 3 decades of logarithmic scattered light intensity.

Table 1

Detector No.	Mean Angle (Deg)	Particle Dia. Resolved (μm)
1	5.8	1.1
2	11.7	1.1,5.0
3	17.5	1.1,5.0
4	23.4	1.1,5.0
5	29.1	1.1,5.0,10.0
6	34.9	1.1,5.0,10.0,15.6
7	40.8	debris,5.0,10.0,15.6,19.5
8	46.6	debris,5.0,10.0,15.6,19.5

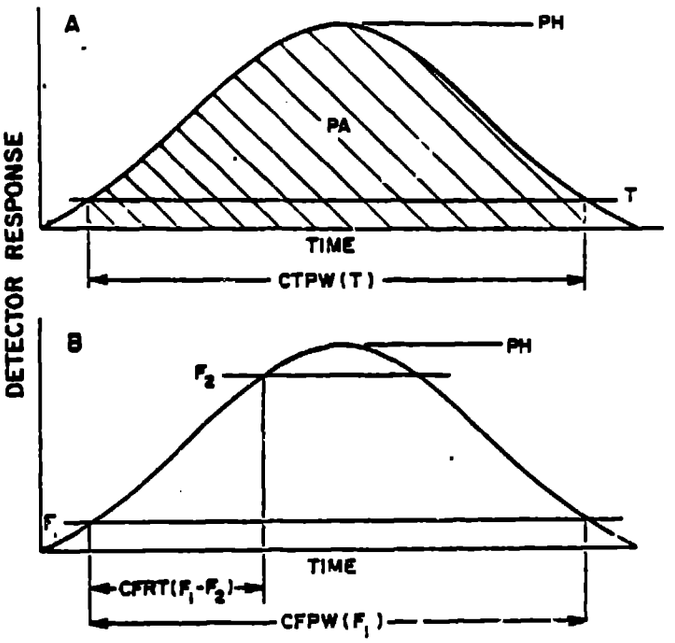

Figure 3. Hypothetical pulse from a detector showing several types of pulse shape measurements. CFRT and CFPW are constant fraction risetime and pulse width, respectively.

PH. CFPW(F1) is the constant fraction pulse width at fraction F1 of the pulse height PH.

To measure these two particle parameters we used an enclosed flow chamber with plane windows in which it is possible to directly measure forward light scatter in the horizontal plane at a polar angle of 0.38 + or - 0.05 degrees using a 1 mm square photodiode detector. The 488 nm laser beam polarization was vertical with a waist of 10.5 μm perpendicular to the flow direction. As test particles we used the same polystyrene latex particles as those described above. Figures 4 and 5 show the data for constant fraction risetime from 10% to 90% of the pulse height, CFRT(10-90), and constant fraction pulse width at 10% of the pulse height, CFPW(10), respectively. The curves in Figures 4 and 5 are from a theoretical model containing no free parameters. In each case the curves were normalized to the data at 5.0 μm by a factor dependent only on amplifier gain and flow velocity. For the limiting case where the particles are much wider than the beam, which is not quite achieved here, the constant fraction risetime and pulse width are directly proportional to particle width in the direction of flow. Details of the theory as well as the design of an electronic module to measure these parameters are given elsewhere.[7]

Conclusion

We have presented two approaches to the measurement of particle size distributions by the detection of light scattered from single particles in a flow photometer. In the first system, the scattered light was detected at multiple angles so that scattering angles could be found where particles with a broad range of sizes could be resolved. In the second

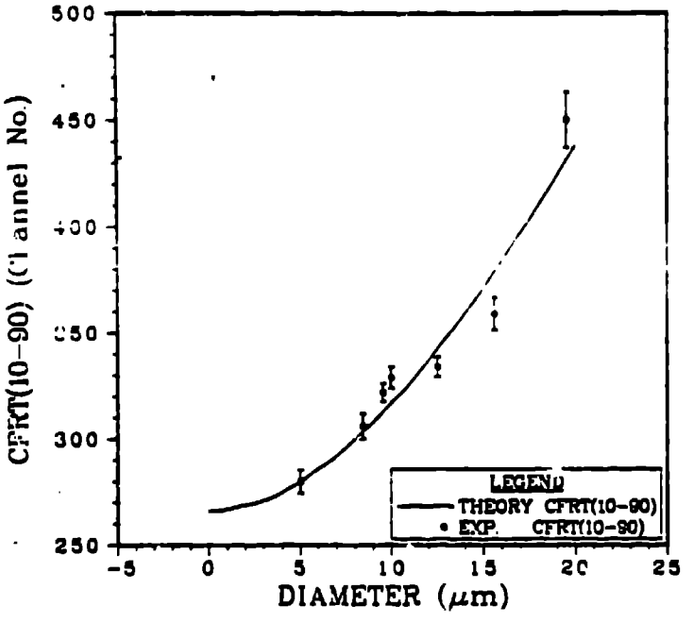

Figure 4. Comparison between theory and experiment for constant fraction risetime measurements between 10% and 90% of the pulse height. The full width of the beam at the e-2 intensity points is 10.5 μm.

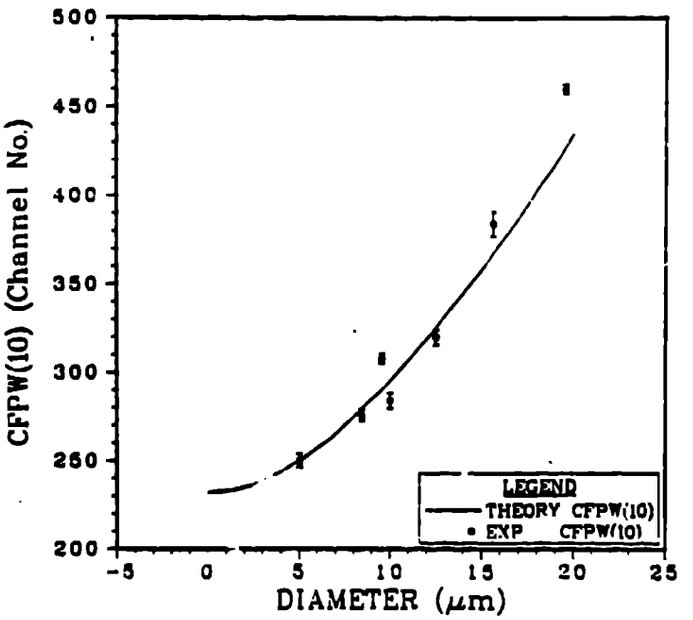

Figure 5. Measurements of constant fraction pulse width at the 10% fraction of the pulse height. Beam width is the same as in Figure 4. The theoretical curves in Figures 4 and 5 were normalized to the data at the 5 μm diameter point.

approach the shape of the detected forward scattered light pulse created as the particle passed through the beam was used for size analysis. Flow system approaches to particle size analysis present an opportunity to obtain rapidly a large amount of information about each particle.

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